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·临床研究·

经内镜逆行胰胆管造影术后胰腺炎患者外周血TFEB、TREM-1表达与病情的关系

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[摘 要] **目的** 探讨行内镜逆行胰胆管造影术(ERCP)后胰腺炎(PEP)患者外周血单个核细胞中转录因子EB(TFEB)、髓样细胞触发受体-1(TREM-1)表达与病情程度的关系。**方法** 选取2019年6月至2022年3月在本院就诊的PEP患者105例为观察对象,设为PEP组,根据患者病情严重程度将其分为轻度组(62例)、中度组(32例)和重度组(11例);选择同期ERCP后未发生PEP患者110例作为对照组。实时荧光定量PCR法检测患者手术前后外周血单个核细胞中TFEB、TREM-1表达水平;采用免疫比浊法检测手术前后血清C反应蛋白(CRP)水平;采用ELISA法检测血清白细胞介素-1(IL-1)、白细胞介素-6(IL-6)水平。术后对患者进行急性生理健康与慢性疾病(APACHE Ⅱ)评分;*Spearman*法分析PEP患者外周血TFEB、TREM-1表达水平与APACHE Ⅱ评分的相关性;*Pearson*法分析PEP患者外周血TFEB、TREM-1表达水平与CRP、IL-1、IL-6的相关性。**结果** PEP组术后血清CRP、IL-1、IL-6水平显著高于术前,且PEP组高于对照组($P<0.05$)。与术前比较,PEP组术后外周血TFEB表达水平显著降低,TREM-1表达水平显著升高($P<0.05$);与对照组比较,PEP组术后外周血TFEB表达水平显著降低,TREM-1表达水平显著升高($P<0.05$)。与轻度组比较,中度组、重度组PEP患者术后外周血TFEB表达水平显著降低,TREM-1表达水平及APACHEⅡ评分显著升高($P<0.05$);与中度组比较,重度组PEP患者术后外周血TFEB表达水平显著降低,TREM-1表达水平及APACHEⅡ评分显著升高($P<0.05$)。相关性分析结果显示,外周血TFEB表达水平与APACHEⅡ评分、血清CRP、IL-1、IL-6水平呈负相关($P<0.05$),外周血TREM-1表达水平与APACHEⅡ评分、血清CRP、IL-1、IL-6水平呈正相关($P<0.05$)。**结论** PEP患者外周血单个核细胞中TFEB表达水平降低,TREM-1表达水平升高,TFEB、TREM-1与患者病情严重程度均相关,可用于评估PEP病情。

[关键词] 内镜逆行胰胆管造影术;胰腺炎;转录因子EB;髓样细胞触发受体-1;外周血单个核细胞
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Relationship between the expression of TFEB and TREM-1 in peripheral blood of patients with pancreatitis after endoscopic retrograde cholangiopancreatography and their condition

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Abstract: Objective To investigate the relationship between the expression of transcription factor EB (TFEB) and triggering receptor expressed on myeloid cells-1 (TREM-1) in peripheral blood mononuclear cells of patients with pancreatitis (PEP) after endoscopic retrograde cholangiopancreatography (ERCP) and the severity of the disease. **Methods** A total of 105 PEP patients who were treated in our hospital from June 2019 to March 2022 were selected as the observation objects and set as the PEP group. According to the severity of the patients' conditions, they were divided into the mild group (62 cases), the moderate group (32 cases) and the severe group (11 cases). A total of 110 patients without PEP after ERCP during the same period were selected as the control group. The expression levels of TFEB and TREM-1 in peripheral blood mononuclear cells before and after surgery were detected by Real-time fluorescence quantitative PCR. The levels of serum C-reactive protein (CRP) before and after surgery were detected by immunoturbidimetry. The levels of serum interleukin-1 (IL-1))and interleukin-6 (IL-6) were detected by ELISA. The acute physiology and chronic health evaluation Ⅱ (APACHE Ⅱ) score was performed on the patients after operation. *Spearman* method was used to analyze the correlation between the expression levels of TFEB and TREM-1 in peripheral blood of PEP patients and the APACHE Ⅱ score. *Pearson* method was used to analyze the correlation between the expression levels of TFEB and TREM-1 in peripheral blood of PEP patients and CRP, IL-1 and IL-6. **Results** The levels of serum CRP, IL-1 and IL-6 after operation in the PEP group were significantly higher than those before operation, and those in the PEP group were higher than those in the control group ($P<0.05$). Compared with before operation, the expression level of TFEB in peripheral blood after operation in the PEP group was significantly decreased, and the expression level of TREM-1 was significantly increased ($P<0.05$). Compared with the control group, the expression level of TFEB in peripheral blood after operation in the PEP group was significantly decreased, while the

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expression level of TREM-1 was significantly increased (P<0.05). Compared with the mild group, the expression levels of TFEB in peripheral blood of PEP patients after operation in the moderate group and the severe group were significantly decreased, while the expression levels of TREM-1 and APACHE Ⅱ score were significantly increased (P<0.05). Compared with the moderate group, the expression level of TFEB in peripheral blood of PEP patients after operation in the severe group was significantly decreased, while the expression level of TREM-1 and APACHE Ⅱ score were significantly increased (P<0.05). The results of correlation analysis showed that the expression level of TFEB in peripheral blood was negatively correlated with APACHE Ⅱ score, serum CRP, IL-1 and IL-6 levels (P<0.05), while the expression level of TREM-1 in peripheral blood was positively correlated with APACHE Ⅱ score, serum CRP, IL-1 and IL-6 levels (P<0.05). **Conclusion** The expression level of TFEB in peripheral blood mononuclear cells of PEP patients is decreased, while the expression level of TREM-1 is increased. TFEB and TREM-1 are related to the severity of the patients′ disease, which can be used to evaluate the condition of PEP.

Keywords: endoscopic retrograde cholangiopancreatography; pancreatitis; transcription factor EB; triggering receptor expressed on myeloid cells-1; peripheral blood mononuclear cells

经内镜逆行胰胆管造影术(endoscopic retrograde cholangiopancreatography,ERCP)是胆胰疾病临床诊断和治疗的重要手段,ERCP后胰腺炎(post-ERCP pancreatitis,PEP)是ERCP术后不良事件,发生率约9.7%,约5%的患者发展为严重病情,影响预后,因此早期诊断PEP并对病情进行评估,有助于改善患者预后[1-2]。转录因子EB(transcription factor EB,TFEB)是自噬和溶酶体生物发生的主要转录因子,可调节免疫炎症反应,介导炎症因子的转录,与感染性疾病有关[3]。有研究显示,TFEB下调可减少溶酶体生物发生,导致小鼠胰腺自噬不足,增加胰腺损伤,诱导实验性胰腺炎发生[4]。髓样细胞触发受体-1(triggering receptor expressed on myeloid cells-1,TREM-1)是免疫球蛋白超家族活化受体,可诱导机体炎症因子表达,是早期炎症活动的标志物[5]。有研究显示,急性胰腺炎患者外周血TREM-1表达水平升高,与患者病情严重程度相关[6]。基于以上研究,推测TFEB、TREM-1与PEP有关,因此,本研究探讨TFEB、TREM-1表达与PEP患者病情程度的关系,以期为PEP的诊治提供参考。

1 资料与方法

1.1 临床资料

选取2019年6月至2022年3月在本院就诊的ERCP后PEP患者105例为观察对象,设为PEP组,其中男62例,女43例;年龄41~80岁,平均(63.20±11.70)岁。根据患者病情严重程度[7]将其分为轻度组(62例)、中度组(32例)和重度组(11例)。纳入标准:①首次接受ERCP治疗;②PEP诊断符合亚特兰大国际共识[7];③临床资料完整,且在术后24 h进行急性生理健康与慢性疾病(acute physiology and chronic health evaluation Ⅱ,APACHEⅡ)评分。排除标准:①合并急慢性胰腺炎;②胆道梗阻、排泄障碍;③心、肝、肾功能不全;④合并胰腺肿瘤。选择同期ERCP后未发生PEP患者110例作为对照组,其中男68例,女42例;年龄40~78岁,平均(64.10±11.50)岁。2组患者性别、年龄比较差异无统计学意义(P>0.05)。本研究经过本院医学伦理委员会批准(190741),患者均签署对本研究的知情同意书。

1.2 各项指标水平检测

采用实时荧光定量PCR法检测患者手术前后外周血单个核细胞中TFEB、TREM-1表达水平。采用免疫比浊法检测手术前后的血清C反应蛋白(C-reactive protein,CRP)水平;采用ELISA法检测血清白细胞介素-1(interleukin-1,IL-1)、白细胞介素-6(interleukin-6,IL-6)水平。

1.3 统计学分析

采用SPSS 22.0统计学软件进行数据分析,符合正态分布的计量资料以均数±标准差($\bar{x}\pm s$)表示,两组间比较采用独立样本t检验,多组间比较使用单因素方差分析,组间两两比较采用*LSD-t*检验,*Spearman*法分析PEP患者外周血TFEB、TREM-1表达水平与APACHE Ⅱ评分的相关性,*Pearson*法分析PEP患者外周血TFEB、TREM-1表达水平与CRP、IL-1、IL-6的相关性,P<0.05为差异有统计学意义。

2 结果

2.1 PEP组与对照组血清CRP、IL-1、IL-6水平比较

PEP组与对照组术前血清CRP、IL-1、IL-6水平比较差异均无统计学意义(P>0.05),PEP组术后血清CRP、IL-1、IL-6水平显著高于术前(P<0.05),且PEP组高于对照组(P<0.05),见表1。

2.2 PEP组与对照组外周血TFEB、TREM-1水平比较

PEP组与对照组术前外周血TFEB、TREM-1表达

水平比较差异均无统计学意义(P>0.05);与术前比较,PEP组术后外周血TFEB表达水平显著降低,TREM-1表达水平显著升高(P<0.05);与对照组比较,PEP组术后外周血TFEB表达水平显著降低,TREM-1表达水平显著升高(P<0.05),见表2。

表1 PEP组与对照组血清CRP、IL-1、IL-6水平比较($\bar{x}\pm s$)

组别	n	CRP(mg/L)		IL-1(pg/mL)		IL-6(pg/mL)	
		术前	术后	术前	术后	术前	术后
对照组	110	23.41±6.56	21.95±6.10	73.44±14.56	70.86±16.47	89.57±16.83	85.21±17.32
PEP组	105	24.58±7.34	47.63±10.21*	74.58±15.34	135.63±26.78*	86.06±15.92	143.26±20.24*
t		1.234	22.506	0.559	21.468	1.569	22.629
P		0.219	0.000	0.577	0.000	0.118	0.000

*:与术前比较,P<0.05

表2 PEP组与对照组外周血TFEB、TREM-1水平比较($\bar{x}\pm s$)

组别	n	TFEB		TREM-1	
		术前	术后	术前	术后
对照组	110	1.03±0.12	1.05±0.15	1.01±0.13	0.98±0.11
PEP组	105	1.05±0.15	0.65±0.10*	1.03±0.12	2.28±0.33*
t		1.082	22.896	1.171	39.107
P		0.280	0.000	0.243	0.000

*:与术前比较,P<0.05

2.3 不同严重程度PEP患者术后外周血TFEB、TREM-1水平及APACHE Ⅱ评分比较

与轻度组比较,中度组、重度组PEP患者术后外周血TFEB表达水平显著降低,TREM-1表达水平及APACHE Ⅱ评分显著升高(P<0.05);与中度组比较,重度组PEP患者术后外周血TFEB表达水平显著降低,TREM-1表达水平及APACHE Ⅱ评分显著升高(P<0.05),见表3。

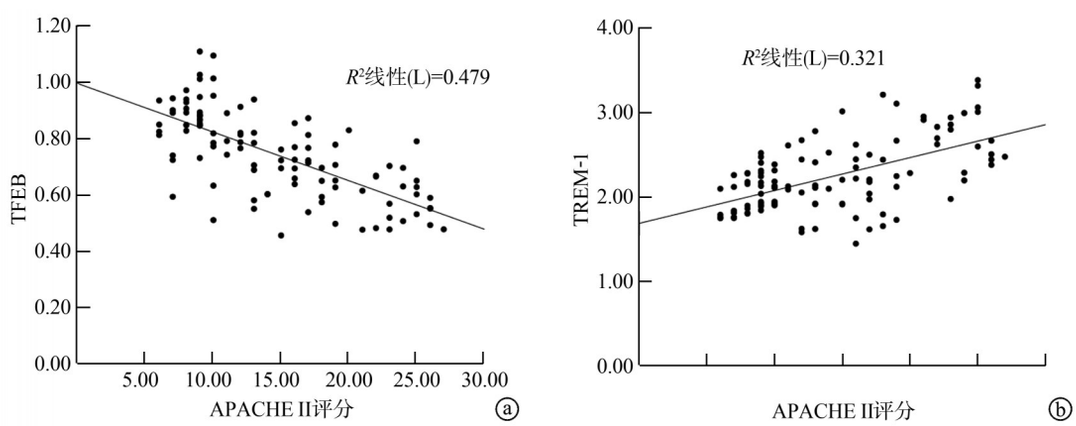
表3 不同严重程度PEP患者术后外周血TFEB、TREM-1水平及APACHE Ⅱ评分比较($\bar{x}\pm s$)

组别	n	TFEB	TREM-1	APACHE Ⅱ(分)
轻度组	62	0.70±0.08	2.12±0.24	8.76±1.78
中度组	32	0.61±0.10*	2.45±0.30*	16.22±2.56*
重度组	11	0.50±0.05*#	2.68±0.23*#	22.18±3.72*#
F		31.878	31.887	223.180
P		0.000	0.000	0.000

*:与轻度组比较,P<0.05;#:与中度组比较,P<0.05

2.4 PEP患者外周血TFEB、TREM-1水平与APACHE Ⅱ评分的相关性

相关性分析结果显示,外周血TFEB表达水平与APACHE Ⅱ评分呈负相关(r=−0.642,P<0.05),外周血TREM-1表达水平与APACHE Ⅱ评分呈正相关(r=0.530,P<0.05),见图1。



a:TFEB与APACHE Ⅱ评分的相关性;b:TREM-1与APACHE Ⅱ评分的相关性

图1 PEP患者外周血TFEB、TREM-1表达水平与APACHE Ⅱ评分的相关性

2.5 PEP患者外周血TFEB、TREM-1水平与CRP、IL-1、IL-6的相关性

外周血TFEB表达水平与血清CRP、IL-1、IL-6水平呈负相关(P<0.05),外周血TREM-1表达水平与血清CRP、IL-1、IL-6水平呈正相关(P<0.05),见表4。

表4 PEP患者外周血TFEB、TREM-1水平与CRP、IL-1、IL-6相关性

指标	TFEB		TREM-1	
	r	P	r	P
CRP	−0.523	<0.001	0.478	<0.001
IL-1	−0.395	<0.001	0.551	<0.001
IL-6	−0.603	<0.001	0.567	<0.001

3 讨论

TFEB参与溶酶体到细胞核的信号转导,其磷酸化水平可调节转录后水平,哺乳动物雷帕霉素靶蛋白(mammalian rapamycin target protein,mTOR)和细胞外调节蛋白激酶(extraeellular signal regulated protein kinase,ERK)/丝裂原活化蛋白激酶(mitogen-activated protein kinase,MAPK)在丝氨酸142和丝氨酸211处可使TFEB磷酸化并导致其滞留胞浆,从而使TFEB转录活性失活,但不影响TFEB在细胞核的分布。在细胞核中,TFEB通过调控mTOR通路自噬相关基因表达,增强自噬,参与自身免疫性疾病、炎症损伤等的发生发展[8]。急性胰腺炎与受损的腺泡细胞自噬通量、细胞内酶原活化、细胞坏死和炎症有关,研究显示,TFEB在酒精诱导的小鼠胰腺炎模型中表达水平降低,而过表达TFEB可改善胰腺损伤,其机制与酒精诱导可使TFEB介导的溶酶体生物合成受损导致自噬不足,从而导致胰腺损伤有关[9]。另有研究显示,α7烟碱型乙酰胆碱受体激活通过增强TFEB介导的腺泡细胞自噬来保护实验性胰腺炎损伤[10]。还有研究显示,TFEB可增强溶酶体功能,减少促炎细胞因子分泌,TFEB上调可降低IL-1、IL-6等炎症因子释放,调节自噬相关基因表达,促进线粒体自噬,保护脂多糖诱导的肺组织和肺泡上皮细胞的炎症损伤[11-12]。本研究结果显示,PEP患者术后外周血TFEB表达水平显著降低,且随着病情加重,降低越显著,提示TFEB与PEP的发生发展有关,推测其可能的原因:在正常情况下,自噬对维持胰腺腺泡细胞内稳态具有重要作用[13],而PEP是手术后引发的胰腺损伤,可能与TFEB介导的胰腺自噬障碍有关,TFEB表达水平降低引发自噬不足,导致腺泡细胞严重变性,引发胰腺炎。

TREM-1是免疫球蛋白超家族成员,主要表达于中性粒细胞、单核巨噬细胞表面,TREM-1可放大Toll样受体介导的炎症反应,促进肿瘤坏死因子-α(tumor necrosis factorα,TNF-α)、IL-1等炎症因子释放,在炎症性疾病中发挥重要作用[14]。TREM-1还以可溶性形式存在,在血清、胸水、肺泡灌洗液中均被检出[15]。在重症中暑大鼠急性肺损伤中,抑制TREM-1表达,可抑制TNF-α、IL-6和IL-1β的释放,减轻大鼠的急性肺损伤[16]。在慢性阻塞性肺病(chronic obstructive pulmonary disease,COPD)小鼠模型中,TREM-1呈高表达,抑制TREM-1可有效改善COPD小鼠肺组织损伤,减少巨噬细胞浸润,其作用机制可能与抑制NOD样受体蛋白3炎性体的激活和细胞焦亡有关[17]。有研究显示,TREM-1在急性重症胰腺炎患者外周血中表达水平升高,对病情的评估有一定价值[18]。本研究结果显示,PEP患者外周血TREM-1表达水平显著升高,与患者病情严重程度有关,推测其可能的原因是PEP患者在手术过程中由于受到刺激,引发机体免疫反应,TREM-1水平升高,促进炎症因子释放,加重胰腺炎性损伤。另外,本研究还发现,PEP患者血清CRP、IL-1、IL-6水平升高,且TFEB表达水平与APACHE Ⅱ评分、血清CRP、IL-1、IL-6水平呈负相关,TREM-1与APACHE Ⅱ评分、血清CRP、IL-1、IL-6水平呈正相关,提示TFEB、TREM-1可反映患者病情严重程度和炎症因子水平,对患者的病情评估有一定临床意义。

综上所述,PEP患者外周血单个核细胞中TFEB表达水平降低,TREM-1表达水平升高,TFEB、TREM-1与患者病情严重程度均相关,可用于评估PEP病情。

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